Real-World Clinical Performance Evaluation of a Fourth-Generation HIV Antigen/Antibody Differentiation Test

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Background: HIV testing is still an important component of routine sexual health screening, assessment of at-risk individuals and as part of the care of pregnant women. To prevent further transmission of infection, it is important that HIV tests are highly sensitive and that positive cases are not missed. HIV serologic antigen/antibody tests are commonly used as they are capable of detecting recent and established infection.

Methods: In this study we assessed the performance of the Elecsys HIV Duo assay (Elecsys assay) against the Abbott Architect assay in 10 121 samples from US and non-US adult, pediatric, and pregnant populations including low-risk, high-risk, and known positive cohorts. Congruent repeatedly reactive and/or discrepant samples followed a confirmatory algorithm consisting of an antigen/antibody differentiation assay and a nucleic acid test, as per the study protocol.

Results: The overall sensitivity of the Elecsys assay was 100.00% (95% CI 99.81–100.00 [1977/1977]), and the specificity was 99.84% (95% CI 99.73–99.91 [8129/8142]). The Elecsys assay detected all positive samples within the study, including all 50 antigen-only positive samples and samples from different HIV subtypes, including group O, group M subtypes, HIV-2 positives, and HIV-1 and HIV-2 dual positives.

Conclusions: The Elecsys HIV Duo assay was highly sensitive for diagnosis of HIV in a range of clinical samples from the United States and outside the United States and is suitable for routine use.

INTRODUCTION

HIV continues to be a major public health concern, with approximately 38 million people worldwide living with HIV at the end of 2019, and it is estimated that only 81% of people living with HIV know their status (1). HIV is spread through certain bodily fluids; in the United States, this is mainly during risky sexual behaviors or sharing of drug injection equipment (2). Less commonly, transmission is due to accidental contaminated sharps injury or from mother to child during pregnancy, birth, or breastfeeding (2). HIV testing is important both in medical diagnosis and management, particularly of at-risk individuals and pregnant women and as part of routine sexual

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IMPACT STATEMENT

This study demonstrates high sensitivity of the Elecsys HIV Duo assay for use in screening for HIV infection as part of antenatal care, sexual health assessment, and assessment of at-risk individuals. The performance of this test was assessed in a diverse range of testing populations, showing excellent sensitivity of both the antigen and antibody modules for the detection of single- and dual-positive samples.

health screening (3). Additionally, HIV testing forms part of the bloodborne pathogen risk assessment following workplace exposure to potentially infected tissues and body fluids (4), and the assessment of patients prior to commencement of preexposure prophylaxis (5, 6).

One of the early markers of HIV infection is the HIV-1 p24 antigen (Ag), which appears transiently within 2 weeks of infection (7, 8) and before an antibody (Ab) response is detectable (9). Anti-HIV Abs are detected around 2 weeks postinfection depending on the diagnostic method used (7, 10), and the Ab response is typically maintained over the course of infection, although it declines in those receiving antiretroviral therapy (11). The CDC recommends a complex HIV testing algorithm, which consists of an Ag/Ab combination immunoassay followed by a HIV-1/HIV-2 differentiation assay and a nucleic acid test (NAT) if the initial test is reactive (12). However, if the initial test is nonreactive, then no further testing is required, and the individual is deemed HIV negative (12). Hence, high sensitivity of the immunoassays is crucial to avoid any false-negative results.

The combined Ag/Ab immunoassays, also known as fourth-generation HIV tests, reduce the negative test window to around 2 weeks, compared to the 3-week window of third-generation IgG/IgM Ab tests (10). Fourth-generation tests have been shown to accurately detect acute HIV infections and reduce the diagnosis window across a variety of different testing populations, including pregnant women (13–17). Despite fourth-generation tests being able to detect the HIV-1 p24 Ag and the IgG/IgM anti-HIV-1/HIV-2 and group O Abs, many only give a single result and therefore cannot differentiate between Ag and Ab readings (10). However, differentiating HIV Ag vs Ab positivity can help to detect recency of infection (18). In recent years, tests have been developed that can distinguish between HIV-1 Abs, HIV-2 Abs, and the p24 Ag (10). The Bio-Rad BioPlex 2200 HIV Ag-Ab assay was the first assay approved by the US Food and Drug Administration (FDA) to report the detection of HIV-1 p24 Ag, anti-HIV-1 (including group M subtype), and anti-HIV-2 Abs independently (19).

The Roche Elecsys HIV Duo assay (Elecsys assay) can provide separate Ag and Ab determinations in parallel, using a 2-incubation step sandwich immunoassay with a rapid test time of 18 min when used on the cobas® e 801 immunoassay analyzer (Roche Diagnostics, Penzberg, Germany). The Elecsys assay is designed for the detection of HIV-1 p24 Ag as well as Abs to HIV-1 and HIV-2 with separate determinations. The Elecsys assay uses monoclonal Abs to detect HIV-1 p24 Ag and recombinant Ags derived from the Env and Pol regions of HIV-1 gp41 (including group O) and the HIV-1 reverse transcriptase and HIV-2 (gp36 and HIV-2 reverse transcriptase) to detect HIV-specific Abs. Previous studies using the Elecsys assay have shown sensitive early detection of HIV in samples from 5 international centers (20) and demonstrated detection of HIV in the Chinese population with multiple HIV-1 genotypes (21).

The objective of this study was to assess the clinical performance of the Elecsys assay using the cobas e 801 immunoassay analyzer at 3 US testing sites using serum and plasma samples from US and non-US adult, pediatric, and pregnant populations, including low-risk, high-risk, and known positive cohorts.

MATERIALS AND METHODS

Study Design and Ethics

This was a multicenter study, including both prospective analysis of samples for routine clinical testing and archived samples, with certificates of analysis, that were previously collected during routine clinical practice or were commercially acquired. There were 20 collection sites for prospective samples consisting of 6 vendors providing archived specimens and 1 vendor and 13 sites for prospective collection of fresh specimens. The testing sites comprised 3 cobas testing sites and 3 confirmatory laboratories (2 US and 1 non-US).

All prospectively collected subjects provided signed informed consent. Archived samples included those where informed consent was previously collected prior to archiving. Ethical approval was provided by either the Institutional Review Board or ethics committee associated with each participating site (IRB: 00000533 [for 11 sites]; Federal Wide Assurance FWA00003937; FWA00005367; FWA00003007; FWA00004495).

Samples

Samples were all classified as high or low risk of HIV (definitions described in the following discussion), negative for HIV, or known positive (previously positive by western blot, NAT, or differentiation assay), and collected from US and non-US sources. Serum and plasma samples were collected to validate that the Elecsys assay can be performed using either matrix. All methods used in the testing algorithm (with the exception of the Abbott RealTime HIV-1 NAT) could use serum or plasma interchangeably. The Abbott RealTime HIV-1 NAT required a plasma sample. All NAT testing was performed on a fresh, untested tube where possible.

Patient participation in the study was sample collection during a single visit. Fresh aliquots were used for testing methods; however, this was not always possible (e.g., for pediatric samples where volume was limited). For low-risk samples, at least 1 aliquot was tested fresh, and all testing (initial and repeat if required) was performed within 72 h. For high-risk and known HIV-positive samples, all aliquots were frozen and stored until randomization. Upon randomization, a fresh, untested aliquot was sent to the respective testing site for screening. Any reactive or discrepant samples moved on to confirmation testing, where possible, using a fresh, untested, residual aliquot for each subsequent method.

Clinical samples were collected from low- and high-risk/HIV-positive and HIV-negative pediatric, adult, and pregnant patients. In this study, US adult samples were defined as $\geq$22 years of age. There were no subjects in the study <2 years old. Non-US adult samples obtained from commercial sources were $\geq$18 years of age. Non-US pediatric samples obtained from commercial sources were $\geq$2 years of age. Low-risk adult and pediatric samples were collected from voluntary blood donors, presurgical hospital admissions, routine examination patients, or vendors. Samples from high-risk cohorts were from subjects who had at least 1 of the following risk factors: transfusions recipient, injection drug user, hemodialysis, hemophilia, sexual contact with infected person, high-risk sexual behaviors, occupational exposure, current/past residence in endemic region, previously incarcerated, sexually transmitted disease patients, and sexual abuse by an infected person. Known positive adult and pediatric samples were collected from HIV clinics, routine HIV physical examinations, or vendors. Samples from known positive

pregnant women were collected from HIV clinics, routine obstetrical examinations, or vendors, and patients were distributed between trimesters. Pediatric samples from all 3 cohorts were not included from subjects considered 'a ward of state'.

Samples were also commercially obtained including those from an HIV-2 endemic region that were HIV-2 known positives, HIV-1 group O positive, HIV-1 group M subtypes (A, B, C, D, CRF01_AE, CRF02_AG, CRF06, CRF11, CRF14, CRF18, CRF36) HIV-1 Ag and Ab positives, and HIV-1 Ag positive and Ab negatives.

Detection of different subgroups was also assessed using known positive viral lysates, spiked into negative serum and naïve samples using viral lysates from HIV-1 group M (CFR06, CFR11, CRF14, CRF18, CRF36); HIV-1 groups N, O, and P positives; and HIV-2 group A and B positives. In total, 37 viral lysates were obtained from the Max v. Pettenkofer Institute, Germany and the University of Rouen, France. Samples were provided along with the inactivated cell culture supernatant and the full sequence information.

Sample Processing

Serum collection tubes were allowed to clot for at least 60 min (or according to collection tube manufacturing instructions) and then centrifuged. Serum was separated from the cells and placed in aliquot tubes with unique subject ID labels. Plasma collection tubes were centrifuged, and the plasma was removed from the cells and placed in aliquot tubes with a unique subject ID label. K2 EDTA plasma collection tubes were used, with the exception of Ag-positive/Ab-negative samples, which were citrated plasma (specific bleeds from seroconversion panels). Fresh serum and plasma sample aliquots were maintained at 2 °C to 8 °C until transferred to testing sites. Transfer of the testing aliquots occurred within approximately 24 h of collection. Fresh samples had testing completed (including any necessary reruns) within 72 h of sample collection.

The additional aliquots not used for fresh testing were frozen at −20 °C (−15 °C to −25 °C) or lower within 48 h and were stored according to the testing requirements of the reference assays within the testing algorithm. Long-term sample storage was maintained at −70 °C or colder.

For assessment of sensitivity using viral lysates, serial dilutions of inactivated viral culture supernatant spiked into HIV negative serum were prepared and measured. The range of high and near cutoff reactivity was defined by the FDA.

Testing

Samples were tested with the Elecsys assay using the cobas e 801 immunoassay analyzer (total assay time of 18 min), and results were compared with the FDA-approved reference assay, Abbott Architect HIV Ag/Ab Combo (Abbott assay), a fourth-generation test, on the Architect platform (Abbott Laboratories, IL, USA). Confirmatory testing was carried out using either the Bio-Rad BioPlex 2200 HIV-Ag-Ab assay, the Abbott RealTime HIV-1 assay (NAT), or the Roche cobas® HIV-1/HIV-2 qualitative PCR (NAT) test using the cobas® 6800/8800 platform (confirmatory testing depended on the sample type/and whether results were discrepant between tests) (Fig. 1).

If samples were negative with both the Elecsys and Abbott assays, then no further testing was needed. If tests were reactive by either or both assays, then further confirmatory testing was carried out with the Bio-Rad assay. If results were negative using the Bio-Rad assay, then samples were further tested using a NAT (except for HIV-positive samples, previously defined as positive prior to inclusion in this study). If tests were positive with the Elecsys, Abbott, and Bio-Rad assays, then confirmatory testing with a NAT was not required but may have been considered (e.g., if the sample was from a low-risk cohort or to further

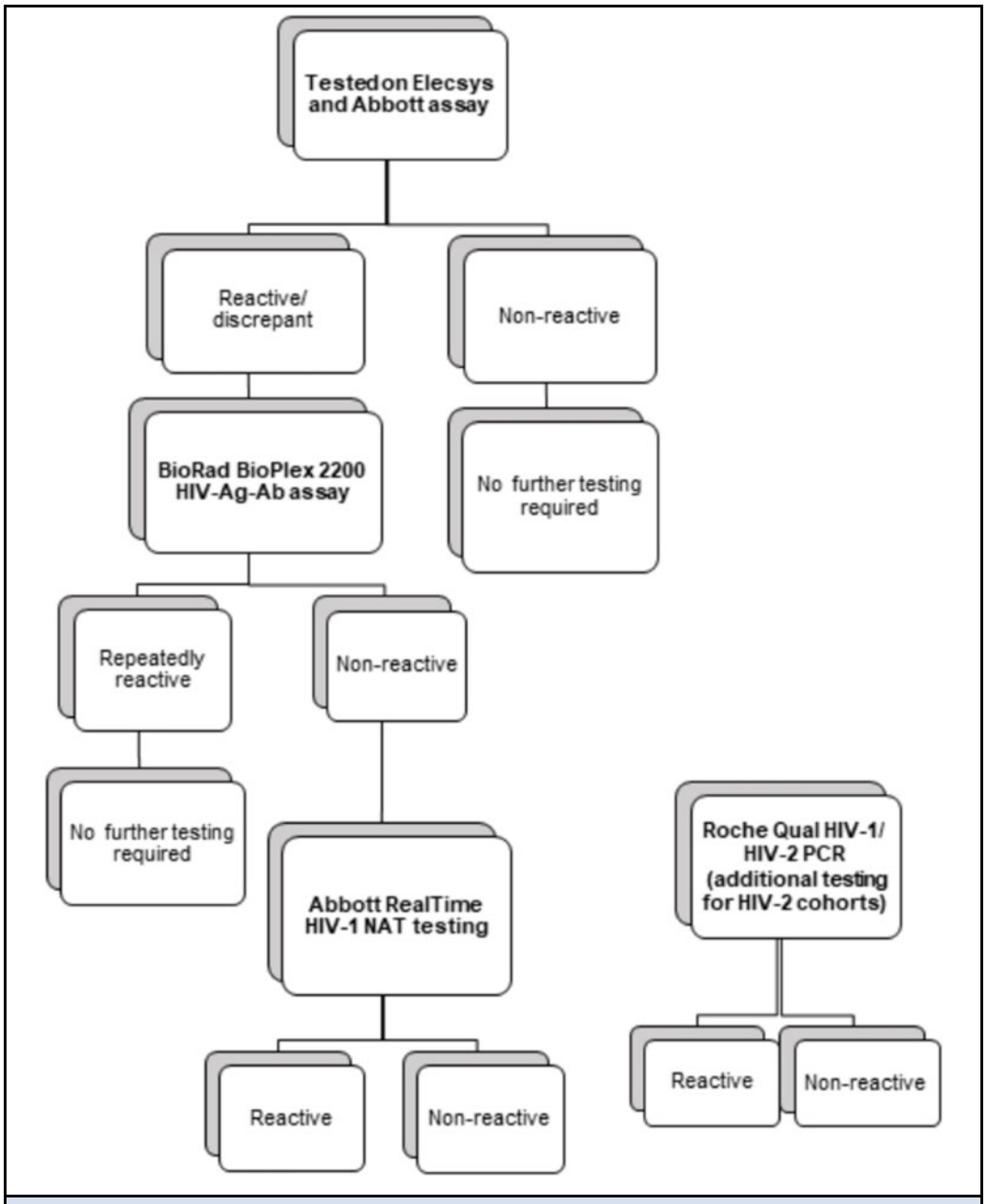

Fig. 1. Testing algorithm used in this study.
All discrepant samples proceeded through the algorithm until there was sufficient evidence of infection.

characterize HIV-2 positive samples) (Fig. 1 and Supplemental Table 1 in the online Data Supplement). Final interpretation of HIV status was determined as sufficient evidence that infection status was achieved (Supplemental Table 2). If final HIV status was still unclear after completing the testing algorithm, samples were sent to Roche Research and Development for

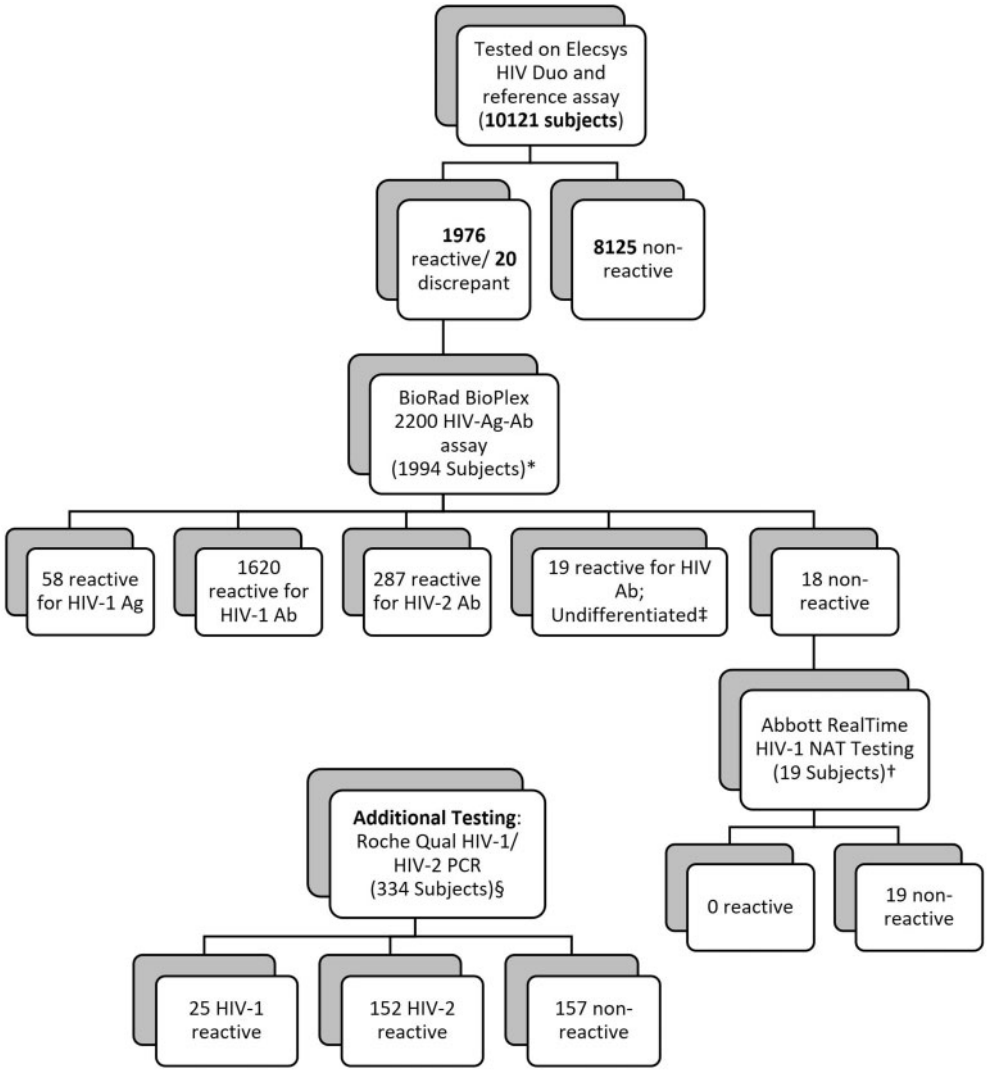

Fig. 2. Sample flow through the study.

*Eight subjects had reactivity for Ag and Ab to HIV, so the total does not reflect the overall number of samples tested. Two subjects had insufficient volume for confirmation testing so their respective certificate of analysis was used for supporting evidence of HIV infection.

†All discrepant samples were to be tested throughout the entire algorithm. Two subjects had insufficient volume for HIV-1 NAT testing. One subject was congruent reactive on Elecsys HIV Duo and the reference assay and non-reactive on the Bio-Rad BioPlex 2200.

‡The undifferentiated samples are those that were reactive on the initial immunoassay and the HIV-1 and HIV-2 Ab differentiation immunoassay, but with HIV differentiation undifferentiated. Specimens that are reactive on the initial combination immunoassay and nonreactive or indeterminate on the HIV-1, HIV-2 differentiation immunoassay were further tested with an FDA-approved NAT test.

§In total, 334 reactive or discrepant samples from HIV-2 cohorts (199 HIV-2 confirmed positives and 135 from an HIV-2 endemic area) were tested to provide additional evidence of single or dual infection for HIV. One subject had insufficient volume for Roche Qual HIV-1/2 PCR testing.

discrepant resolution. When sufficient sample volume was unavailable to complete the testing algorithm/resolve final status, final status was listed as inconclusive.

Analyses

The performance of the Elecsys assay was compared with the final HIV status decided via the testing algorithm for each patient subgroup and for the overall subject population included in this study. Sensitivity, specificity, and 95% CIs are provided for each subgroup, where the sample numbers allow for calculation of these parameters (e.g., specificity is not provided where there are no negative cases within the sample population). Samples with insufficient volume to complete the algorithm were not included in the sensitivity/specificity calculations. Overall, the sensitivity of the assay was determined using HIV-positive samples that were confirmed positive with a Bio-Rad assay or NAT result. Anti-HIV–positive/HIV-Ag–negative samples were used to validate the Ab module of the assay. For the Ag module, HIV-Ag–positive/anti-HIV–negative samples were used for sensitivity calculations.

R version 3.4.0 and SAS version 9.4 software were utilized as a biometric tool for statistical analysis of the data.

RESULTS

Subjects

Overall, 10 121 subjects were recruited and tested; of these, 5828 (57.58%) were female and 4240 (41.89%) were males. For 53 subjects, sex was unknown or not reported (52 samples were from seroconversion panels, which did not provide this information) (Table 1). Subjects were enrolled across the main US race demographic groups; most subjects were either black/African American (29.05%) or white (64.47%) (Table 1). In total, 7121 (70.36%) samples were prospectively collected and 3000 (29.64%) were previously archived samples. Of the total samples, 1273 (12.58%) were obtained from commercial sources.

Comparison of the Overall Performance of the Elecsys and Abbott Assays with the Final Status

In total, 1977 of the 10 121 samples tested were confirmed as HIV positive and 8142 were concluded HIV negative (Fig. 2). Results from 2 subjects that were Elecsys negative/Abbott positive were inconclusive. Final testing showed that the Elecsys assay agreed with the final HIV status in 8129 of the 8142 nonreactive and 1977 of the 1977 reactive samples (Table 2). The overall sensitivity and specificity of the Elecsys assay across all 10 121 samples in this study relative to the final HIV status was 100.00% (95% CI 99.81–100.00 [1977/1977]) and 99.84% (95% CI 99.73–99.91 [8129/8142]), respectively, which was similar to the Abbott assay (Table 2). The Abbott assay had a slightly lower sensitivity of 99.90% (95% CI 99.63–99.97), with 2 confirmed false-negative results (Table 2). However, it is important to note the final status for the 2 nonreactive samples with the Elecsys assay was inconclusive due to insufficient sample to complete the algorithm (Table 3).

Analysis of the Samples Discrepant between the Elecsys and Abbott Assays and the Final Result

In total, 20 samples were discrepant between the Elecsys and Abbott assays, with 14 samples Elecsys assay reactive/Abbott assay nonreactive and 6 samples Abbott assay reactive/Elecsys assay nonreactive (Table 3). Of the 14 samples that were Elecsys assay reactive/Abbott assay nonreactive, 2 samples were confirmed as positive using the Bio-Rad assay. The additional 12 samples were false positive with the Elecsys assay (Table 3). One sample was false positive with both the Abbott and Elecsys assays. Of the 6 samples that were Elecsys assay nonreactive/Abbott assay reactive, 4 were

Table 1. Subject characteristics per enrollment group by sex, age, and race.

	Sex, n (%)			Age	Race, n (%)							
	Female	Male	Overall	Mean age years (SD)	Unknown	American Indian/ Alaskan Native	Asian	Black or African American	Native Hawaiian or Pacific Islander	White	Other	Missing
Low-risk adult	3962 (64.87)	2146 (35.13)	6108	49.0 (15.5)	0	39 (0.64)	160 (2.62)	990 (16.21)	21 (0.34)	4833 (79.13)	53 (0.87)	58 (0.95)
High-risk adult	203 (40.12)	303 (59.88)	506	43.0 (11.8)	0	6 (1.19)	8 (1.58)	251 (49.60)	6 (1.19)	239 (47.23)	0	0
HIV-1 confirmed positive adult	216 (20.59)	833 (79.41)	1049	47.8 (11.6)	0	6 (0.57)	4 (0.38)	519 (49.48)	3 (0.29)	508 (48.43)	20 (1.91)	0
Low-risk pediatric	357 (59.20)	246 (40.80)	603	15.6 (5.2)	0	4 (0.66)	35 (5.80)	110 (18.24)	2 (0.33)	434 (71.97)	5 (0.83)	21 (3.48)
High-risk pediatric	103 (51.50)	97 (48.50)	200	19.0 (2.2)	0	1 (0.50)	0	28 (14.00)	0	171 (85.50)	0	1 (0.50)
HIV-1 confirmed positive pediatric	22 (43.14)	29 (56.86)	51	12.8 (6.2)	0	0	1 (1.96)	31 (60.78)	0	6 (11.76)	0	13 (25.49)
HIV-negative pregnant women	199 (100)	0 (0)	199	29.1 (5.3)	0	1 (0.50)	5 (2.51)	3 (1.51)	3 (1.51)	190 (95.48)	2 (1.01)	0
High-risk pregnant women (US)	204 (100)	0 (0)	204	29.0 (5.6)	0	1 (0.49)	3 (1.47)	53 (25.98)	2 (0.98)	117 (57.35)	0	28 (13.73)
HIV-positive pregnant women (US)	49 (100)	0 (0)	49	30.1 (5.6)	0	0	2 (4.08)	33 (67.35)	0	7 (14.29)	0	7 (14.29)
HIV-positive pregnant women (Non-US)	10 (100)	0 (0)	10	28.0 (7.5)	0	0	0	0	0	7 (70.00)	3 (30.00)	0
Non-US HIV-1 confirmed positives	111 (55.50)	89 (44.50)	200	39.9 (10.2)	1 (0.50)	0	28 (14.00)	160 (80.00)	0	10 (5.00)	1 (0.50)	0
HIV-2 endemic area	143 (28.60)	357 (71.40)	500	32.7 (11.4)	0	0	0	500 (100)	0	0	0	0
HIV-2 confirmed positive	130 (65.00)	69 (34.50)	200[1]	51.5 (9.6)	0	0	0	100 (50.00)	0	0	0	100 (50.00)
HIV-1 group O	36 (72.00)	14 (28.00)	50	45.5 (13.0)	0	0	0	50 (100)	0	0	0	0
HIV subtypes (group M)	52 (57.78)	38 (42.22)	90	38.6 (9.5)	0	1 (1.11)	20 (22.22)	65 (72.22)	0	3 (3.33)	1 (1.11)	0
HIV Ag positive/Ab positive	31 (62.00)	19 (38.00)	50	45.7 (12.1)	0	0	0	47 (94.00)	0	0	2 (4.00)	1 (2.00)
HIV Ag positive/Ab negative	—	—	52	—	0	0	0	0	0	0	0	52 (100)
Overall	5828 (57.58)	4240 (41.89)	10 121	43.8 (16.9)	1 (0.01)	59 (0.58)	266 (2.63)	2940 (29.05)	37 (0.37)	6525 (64.47)	87 (0.86)	281 (2.78)

For 1 sample, sex was unknown; low-risk pregnant women are included among the low-risk adult cohort. SD, standard deviation.

Table 2. Performance of the Elecsys and Abbott assays vs the final status.

	HIV positive	HIV inconclusive[a]	HIV negative	Total
Elecsys[b]				
Reactive	1977	0	13	1990
Nonreactive	0	2	8129	8131
Abbott[c]				
Reactive	1975	2	5	1982
Nonreactive	2	0	8137	8139
Total	1977	2	8142	10 121

[a] Samples with inconclusive final HIV status were not considered for sensitivity but were counted against the comparison method for specificity.
[b] Elecsys assay sensitivity and specificity (95% CI): 1977/1977 = 100.00% (99.81%–100.00%), 8129/8142 = 99.84% (99.73%–99.91%).
[c] Abbott assay sensitivity and specificity (95% CI): 1975/1977 = 99.90% (99.63%–99.97%), 8137/8144* = 99.91% (99.82%–99.96%).

nonreactive on the Bio-Rad assay and were HIV negative; for 2, the final status was not determined (from the low-risk adult cohort) (Table 3) due to insufficient sample volume for further NAT.

Performance of the Elecsys Assay for the Separate Determination of HIV Ag and Ab

The Elecsys assay was 100% sensitive in both the Ag-positive/Ab-positive and the Ag positive/Ab-negative groups obtained from seroconversion panels and by analysis of the overall study samples (Table 4).

Diagnostic Performance in Different Patient Cohorts and for the Detection of Different HIV Types vs the Final Result/Vendor-Provided Sample Information

The sensitivity of the Elecsys assay across all the sample cohorts within this study vs the final status was 100%, detecting all positive samples (Table 4). The specificity of the Elecsys assay was between 98.92% and 100.00% across all groups; 13 false positives were identified, with the highest number in the low-risk adult (6 samples) and HIV-2 endemic area (4 samples) cohorts (Table 4).

The Elecsys assay was also 100% sensitive across all HIV groups and subtypes in this study (HIV-2, HIV-1 group O, HIV-1 group M [A, B, C, D, CRF01_AE, CRF02_AG, CRF06, CRF11, CRF14, CRF18, CRF36], HIV-1 group N and group P) (Table 5). Additionally, the Elecsys assay identified all HIV-2, HIV-1, and HIV-1/HIV-2 dual infections in samples from HIV-2 endemic regions (Table 5). Spike-in experiments, using a range of HIV-positive viral lysates spiked into HIV-negative samples, showed that the Elecsys assay was able to detect Abs to both HIV types and all genetic subtypes and circulating recombinant forms tested (Table 5).

DISCUSSION

In all the conclusive results in this study, the Elecsys assay displayed 100% sensitivity in detecting HIV cases from large and diverse clinical cohorts from US and non-US samples (with the caveat that 2 samples in this study were inconclusive and were excluded from sensitivity calculations). Subjects represented a broad demographic, reflecting those who may present for routine testing in the United States. This study also demonstrated sensitivity of the Elecsys assay in a more diverse range of HIV groups than has previously been presented (20, 21).

The high sensitivity demonstrated by the Elecsys assay is a crucial characteristic of HIV

Table 3. Analysis and resolution of samples that were discrepant between the Elecsys assay, the Abbott assay, and the final result.

Subject number	Sample cohort	Elecsys assay	Abbott assay	Bio-Rad assay	Abbott RealTime HIV-1 assay	cobas HIV-2 qualitative PCR	Final HIV status	Elecsys assay/Abbott assay outcome
50111	HIV-2 endemic	Reactive	Nonreactive	Nonreactive	Nonreactive	Nonreactive	HIV negative	Elecsys false positive Abbott true negative
50120	HIV-2 endemic	Reactive	Nonreactive	Nonreactive	Nonreactive	Nonreactive	HIV negative	Elecsys false positive Abbott true negative
50117	HIV-2 endemic	Reactive	Nonreactive	Nonreactive	Nonreactive	Nonreactive	HIV negative	Elecsys false positive Abbott true negative
50249	HIV-2 endemic	Reactive	Nonreactive	Nonreactive	Nonreactive	Nonreactive	HIV negative	Elecsys false positive Abbott true negative
D3043	High-risk adult	Reactive	Nonreactive	Reactive	Nonreactive	NT	HIV positive	Elecsys true positive Abbott false negative
D1030	High-risk pediatric	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D3600	Low-risk adult	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D3992	Low-risk pediatric	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D9696	Low-risk adult	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D11623	Low-risk adult	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D11274	Low-risk adult	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D9015	Low-risk adult	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D1075	High-risk pregnant	Reactive	Nonreactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott true negative
D1962	Confirmed positive pediatric	Reactive	Nonreactive	Reactive	QNS	NT	HIV positive	Elecsys true positive Abbott false negative

Continued

Table 3. (continued)

Subject number	Sample cohort	Elecsys assay	Abbott assay	Bio-Rad assay	Abbott RealTime HIV-1 assay	cobas HIV-2 qualitative PCR	Final HIV status	Elecsys assay/Abbott assay outcome
D3669	Low-risk adult	Nonreactive	Reactive	Reactive	QNS	NT	Inconclusive	Inconclusive
D3886	Low-risk pediatric	Nonreactive	Reactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys true negative Abbott false positive
D5125	Low-risk adult	Nonreactive	Reactive	Nonreactive	Nonreactive	NT	Inconclusive	Inconclusive
D4044	Low-risk adult	Nonreactive	Reactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys true negative Abbott false positive
D4794	Low-risk pediatric	Nonreactive	Reactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys true negative Abbott false positive
D7687	Low-risk adult	Nonreactive	Reactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys true negative Abbott false positive
D4241	Low-risk adult	Reactive	Reactive	Nonreactive	Nonreactive	NT	HIV negative	Elecsys false positive Abbott false positive

QNS, quantity not sufficient; NT, not tested due to QNS or not required per the testing algorithm.

Table 4. The performance of the Elecsys assay vs the final status in each of the subject groups within this study.

	Elecsys positive/final status positive (n/n) Sensitivity % (95% CI)	Elecsys negative/final status negative (n/n) Specificity % (95% CI)
HIV Ag and Ab positive samples	50/50	0/0
	100.00% (92.87%–100.00%)	—
HIV Ag positive and Ab negative samples	52/52	0/0
	100.00% (93.12%–100.00%)	—
HIV Ab positive and Ag negative (determined from the cohorts in this study)[a]	1437/1437	0/0
	100.00% (99.73%–100.00%)	—
Low-risk adult	13/13	6087/6093
	100.00% (77.19%–100.00%)	99.90% (99.79%–99.95%)
High-risk adult	12/12	494/494
	100.00% (75.75%–100.00%)	100.00% (99.23%–100.00%)
HIV-1 confirmed positive adult	1049/1049	0/0
	100.00% (99.64%–100.00%)	—
Low-risk pediatric	1/1	601/602
	100.00% (20.65%–100.00%)	99.83% (99.07%–99.97%)
High-risk pediatric	4/4	195/196
	100.00% (51.01%–100.00%)	99.49% (97.17%–99.91%)
Confirmed positive pediatric	34/34	0/0
	100.00% (89.85%–100.00%)	—
HIV-negative pregnant women	0/0	199/199
	—	100.00% (98.11%–100.00%)
High-risk pregnant women (US)	15/15	188/189
	100.00% (79.61%–100.00%)	99.47% (97.06%–99.10%)
HIV-positive pregnant women (US)	49/49	0/0
	100.00% (92.73%–100.00%)	—
HIV-positive pregnant women (non-US)	100.00% (72.25%–100.00%)	—
Non-US HIV-1 confirmed positives	200/200	0/0
	100.00% (98.12%–100.00%)	—
HIV-2 endemic area	131/131	365/369
	100% (97.15%–100%)	98.92% (97.25%–99.58%)
HIV-2 confirmed positive	200/200	0/0
	100.00% (98.12%–100.00%)	—
HIV-1 group O	50/50	0/0
	100.00% (92.87%–100.00%)	—
HIV subtypes (group M)	90/90	0/0
	100.00% (95.91%–100.00%)	—

The data from low-risk pregnant women are included under "low-risk adults."
[a] HIV Ab positive and Ag negative samples confirmed with a reactive Ab result and a nonreactive Ag result using the Bio-Rad assay.

Table 5. The sensitivity of the Elecsys assay for detection of HIV-1 group M subtypes, group N, and group P and HIV-1 and HIV-2 in samples from HIV-2 endemic regions and HIV-positive viral lysates diluted into HIV-negative serum.

Subtype	Number of positives with Elecsys assay/final number of positives	Sensitivity % (95% CI)[a]
A	15/15	100.00% (79.61%–100.00%)
B	15/15	100.00% (79.61%–100.00%)
C	15/15	100.00% (79.61%–100.00%)
D	15/15	100.00% (79.61%–100.00%)
CRF01_AE	15/15	100.00% (79.61%–100.00%)
CRF02_AG	15/15	100.00% (79.61%–100.00%)
CRF06	3/3	
CRF11	1/1	
CRF14	1/1	
CRF18	1/1	
CRF36	1/1	
Group N	1/1	
Group P	1/1	
Samples from HIV-2 endemic regions		
HIV-1 only	26/26	100.00% (87.13%–100.00%)
HIV-2 only	82/82	100.00% (95.52%–100.00%)
HIV-2 group A	2/2	
HIV-2 group B	1/1	
HIV-1/HIV-2 dual infection	13/13	100.00% (77.19%–100.00%)
HIV positive viral lysates diluted into HIV-negative serum		
HIV-1 group M CRF06	4/4	
HIV-1 group M CRF11	1/1	
HIV-1 group M CRF14	1/1	
HIV-1 group M CRF18	1/1	
HIV-1 group M CRF36	1/1	
HIV-1 group N	2/2	
HIV-1 group O	17/17	
HIV-1 group P	1/1	
HIV-2 group A	7/7	
HIV-2 group B	2/2	

[a] Sensitivity and 95% CI data were not calculated for some groups due to the sample size.

diagnostic testing to ensure no false negatives are detected and is especially important due to the testing criteria set by the CDC, which state that only repeatedly reactive samples require additional confirmatory testing with an Ab differentiation immunoassay and, if results are incongruent, with a NAT (12). After further analysis of the 20 samples discrepant between the Abbott

and Elecsys assays, the results show that the Elecsys assay did not display any false-negative results, which agreed with earlier studies that used this assay (20, 21). Conversely, the Abbott assay displayed 2 false-negative results compared to the final HIV status based on confirmatory testing with the fifth-generation Bio-Rad assay and NAT assays. These false negatives were in a high-risk and a pediatric known-positive patient, both of which would likely have been screened using a NAT, according to the CDC guidelines (3). False negatives in a screening situation could be very dangerous as these results would be reported as negative, and no further testing would be conducted, leaving the patients at risk of developing AIDS or further passing on the infection. False negatives have been observed with the Abbott assay previously, suggesting that rarely some cases may be missed (14). These results highlight the need to ensure that high-risk patients are appropriately screened with a NAT or that high-risk status is considered when reviewing any Ab/Ag test results. Conversely, previous studies have highlighted the potential for false-positive test results with the Bio-Rad assay, particularly in low-prevalence testing settings, where positive predictive value can be less than 50% (22, 23). In this study, both the Elecsys and Abbott assays reported false-positive results, and the overlapping CIs of the Elecsys and Abbott assays suggest a similar specificity. In low-prevalence cohorts, a highly specific assay is also important to reduce false positives and the unnecessary burden of repeat testing. There is a careful balance in determining where to set the cutoff for the assay. In the case of HIV infection, a missed diagnosis could lead to delayed care and further spread of infection. As such, the 100% sensitivity of the Elecsys assay in this study is reassuring. The high sensitivity of the Elecsys assay is also comparable to the fifth-generation Bio-Rad assay that previously has been shown to display 100% sensitivity (19). Additionally, the detection of the HIV subtypes by the Elecsys assay, which was also observed in the other studies that used this assay (20, 21), is encouraging given the problems that HIV subtype diversity can cause for accurate diagnosis (24).

The ability of the Elecsys assay to provide independent readings for the Ag and Ab modules allows for the timely diagnosis of recently acquired infections and enables physicians to initiate antiretroviral treatment as early as possible. The Elecsys assay identified 50 out of 50 Ag-positive/Ab-negative samples and all 1437 of the 1437 Ab-positive/Ag-negative samples, accurately identifying patients as singularly positive for Ab/Ag and all subjects in the study who were positive for both. This is in agreement with a previous study using the Elecsys assay, which also demonstrated highly sensitive, distinct identification of each Ag- and Ab-positive result, and high analytical sensitivity for detection of the National Institute for Biological Standards and Control HIV-p24 Ag standard, detecting $\leq$ 1 IU/mL (20). A previous study that assessed the performance of the Bio-Rad assay in identifying acute infections found that the Bio-Rad assay detected about a half of the cases, the majority being Ag positive/Ab negative (25). However, the Bio-Rad assay was less sensitive than a qualitative RNA assay, and increasing viral load positively correlated with Ag detection (25). Thus, the possibility of missing acute infections while following the CDC testing algorithm using these assays should be considered. The consequence of missing acute infections prevents the initiation of early treatment, can increase the likelihood of onward transmission, and can lead to inappropriate use of preexposure prophylaxis that may result in drug resistance (5).

In this study, the Elecsys assay had a high specificity of 99.84%, which was comparable to other studies using the Elecsys assay for diagnosis in

13 328 European samples (99.87%) (20) and 3039 Chinese samples (99.93%) (21). Most of the false-positive results (n = 7) and the 2 inconclusive results were detected in the low-risk cohorts; incidences of false-positive results have also been previously noted for the Bio-Rad assay and Abbott assay (22, 26). This highlights the importance of further testing for all initially reactive samples. While all repeatedly reactive and/or discrepant samples followed the approved testing algorithm for testing confirmation, a limitation of this study may be that not all samples were additionally confirmed using a NAT. However, in a real-world clinical setting, an initially reactive Ab test that was then positive using a HIV differentiation Ag/Ab assay would not need confirmation with a NAT.

In conclusion, the Elecsys assay, reporting separate results for HIV Ag and anti-HIV Abs, displays high sensitivity and specificity, importantly detecting no false-negative results, and therefore is suitable for use in the diverse clinical testing settings in the United States.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: Ab, antibody; Ag, antigen; FDA, Food and Drug Administration; NAT, nucleic acid test.

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